Improving Resolution and Sample Throughput For Protein Analysis Using TSKgel® UP-SW3000 Columns

TSKgel
APPLICATION NOTE

Introduction

Size Exclusion Chromatography (SEC) is an important method for monitoring the impurities of biotherapeutic drugs, in order to minimize the immunologic effect that may occur when such impurities are present. The SEC method is widely used to determine the monomers, dimers, aggregates, and fragments in biotherapeutic drug samples, including monoclonal antibodies (mAbs). A few of the requirements for a successful analysis include: superior resolution, excellent reproducibility and short analysis time. In order to achieve these, an SEC column must have the appropriate particle size and pore size, good bonding chemistry, and suitable column dimensions. To ease method development, SEC columns can be run using an established operating method with minimal time spent on method optimization. To enhance cost-saving measures, these columns should be packed such that they can be operated with both HPLC and UHPLC systems.

This application describes the use of TSKgel UP-SW3000 SEC columns for the analysis of proteins. These columns have a particle size of 2 µm, and are 25 nm in size. The particles are coated with a hydrophilic diol-type bonded phase in order to minimize the interaction between the silica surface and proteins. These columns are designed to be operated with a simple and well established method (sodium phosphate mobile phase, pH 6.8) and are packed for use in both HPLC and UHPLC systems. A comparasion study between a TSKgel UP-SW3000 column and a larger particle sized SEC column (when operated under the same mobile phase conditions with a slight adjustment of the flow rate) show that the TSKgel UP-SW3000 column has a higher resolution while the retention time profile of the separation remains nearly unchanged. Results from the shorter column dimension, 4.6 mm ID × 15 cm, show that the run time can be completed 2 times faster than its longer column dimension counterpart without compromising resolution and reproducibility.

Experimental HPLC Conditions

Columns:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.8 + 100 mmol/L sodium sulfate + 0.05% sodium azide
Gradient:	Isocratic
Flow rate:	0.35 mL/min for TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm and 4.6 mm ID × 15 cm columns 1.0 mL/min for TSKgel G3000SW$_{XL}$, 5 µm, 7.8 x 30 cm column
LC system:	Agilent 1100 Series LC system
Detection:	UV @ 280 nm
Temperature:	25 ºC
Injection vol.:	5 µL for TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm and 4.6 mm ID × 15 cm columns 10 µL for TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column
Samples:	QC protein standard test mixture mAb sample

Results

Figure 1 shows the protein standard calibration curve data that was generated using the TSKgel UP-SW3000 column. The column was run with a simple aqueous mobile phase (sodium phosphate buffer, pH 6.8) as typically reported in literature for SEC separations. The data demonstrates that the TSKgel UP-SW3000 column has a broad and linear resolving range of molecular weights. The shallow slope around the molecular weight of γ-globulin suggests that the particles of the column have an optimized pore size for the separation of proteins with a molecular weight of approximately 150 kDa.

Figure 1. Standard calibration curve of QC protein standard mixture generated by TSKgel UP-SW3000, 4.6 mm ID × 30 cm column

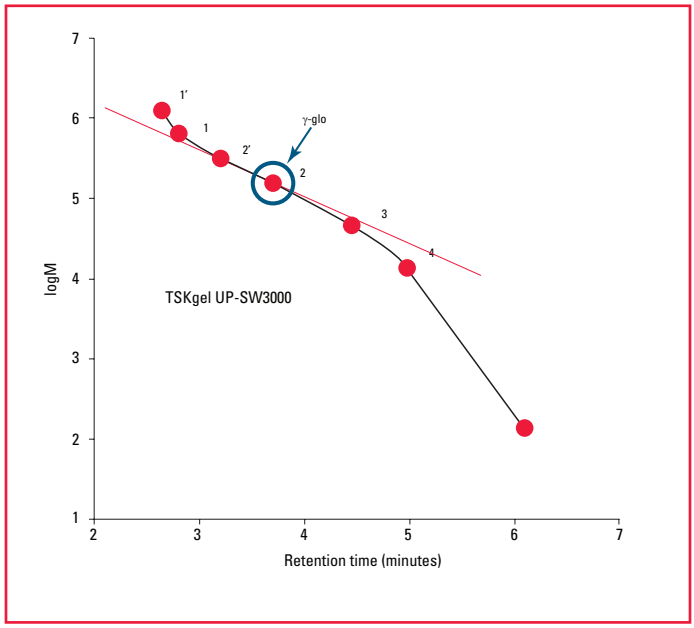


Figure 2 shows comparison data between a TSKgel UP-SW3000 column *(Figure 2, top)* and a 5 µm particle sized SEC column (TSKgel G3000SW$_{XL}$, 7.8 mm ID × 30 cm) *(Figure 2, bottom)* operated under the same mobile phase conditions with a slight change in flow rate. The results indicate that the TSKgel UP-SW3000 provided a major improvement in peak resolution *(Table 1)* and peak shape, while the peak retention times remained nearly unchanged. This suggests that the TSKgel UP-SW3000 column can be compatible with the established protocol that was used for a larger particle sized SEC column, such as a TSKgel G3000SW$_{XL}$ column, with a slight change in flow rate. Since the TSKgel UP-SW3000 column generated a backpressure of only 26.7 MPa at a 0.35 mL/min flow rate, these columns can be used with both HPLC and UHPLC systems.

Figure 2. Comparison study between TSKgel UP-SW3000, 4.6 mm ID × 30 cm and TSKgel G3000SW$_{XL}$, 7.8 mm ID × 30 cm columns using the same mobile phase condition for the separation of a QC protein standard mixture.

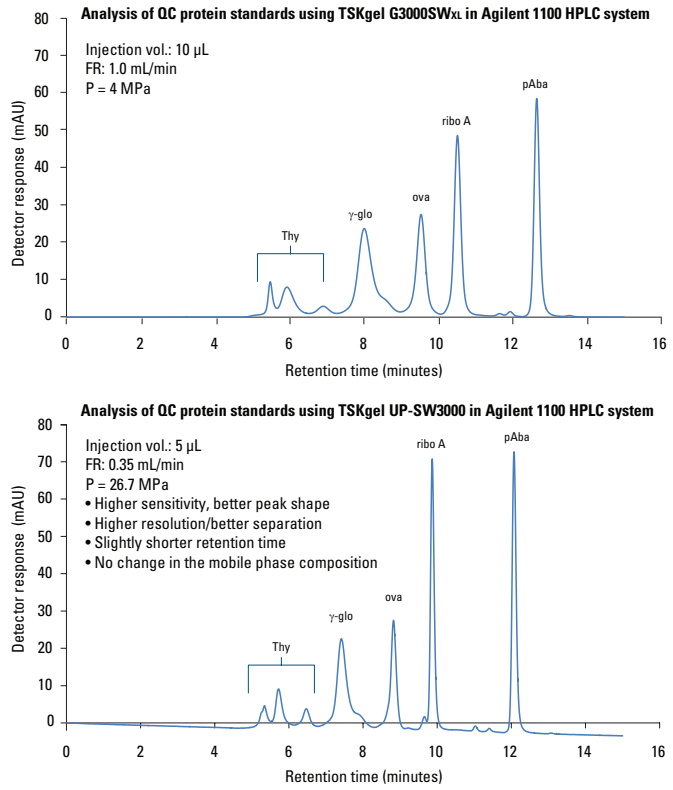
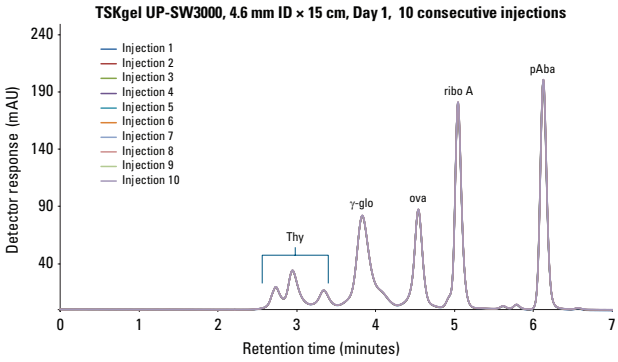


Table 1. Comparison data of peak resolution using QC protein standard mixture (from Figure 2) between TSKgel G3000SW$_{XL}$ and TSKgel UP-SW3000 columns on 1100 series HPLC system

Peak resolution	Rs 1-2	Rs 2-3	Rs 3-4	Rs 4-5	Rs 5-6	Rs 6-7
TSKgel G3000SW$_{XL}$	0.98	1.46	1.56	2.52	2.71	6.41
TSKgel UP-SW3000	0.89	1.68	1.94	3.52	3.35	8.75

Figure 3 shows the elution profile of a QC protein standard mixture generated using a TSKgel UP-SW3000, 4.6 mm ID × 15 cm column operated at 0.35 mL/min. The separation was completed in only 7 minutes, nearly 2 times faster than the same column of 4.6 mm ID × 30 cm dimension. The peak resolution profile remained nearly unchanged. In particular, the resolution profile of thyroglobulin was similar to the resolution that was obtained by the 4.6 mm ID × 30 cm column. This suggests that the separation of high order molecular weight species, such as aggregates from mAb, can be easily achieved using this 4.6 mm ID × 15 cm column. 10 consecutive runs yielded excellent reproducibility. In fact, peaks from 10 consecutive injections were nicely overlaid.

Figure 3. 10 consecutive injections of QC protein standard mixture generated using a TSKgel UP-SW3000, 4.6 mm ID × 15 cm column.



Results from *Table 2* show low percentage relative standard deviation of the theoretical plates from a wide range of molecular weights within 10 consecutive injections of protein standard *(from Figure 2)*. The data suggests that the TSKgel UP-SW3000 columns have a high reproducibility of injection after injection.

Table 2. Reproducibility-Theoretical plates of pAba, γ-globulin and thyroglobulin over 10 consecutive injections using TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm column.

Injection #	pAba-theoretical plates (N)	γ-globulin-theoretical plates (N)	Thyroglobulin-theoretical plates (N)
1	252743	2533	2260
2	25300	2541	2278
3	25397	2541	2281
4	25519	2555	2299
5	25558	2557	2306
6	25567	2554	2302
7	25550	2556	2304
8	25529	2561	2304
9	25521	2558	2303
10	25502	2554	2307
Average	25468.6	2551.0	2297.4
%RSD	0.451	0.362	0.691

Conclusion

The above results demonstrate the broad and linear molecular weight resolving range of TSKgel UP-SW3000 columns. This, in turn, drives the accuracy, reliability and reproducibility for molecules of interest such as the monomer, dimer, and aggregates of mAbs. The comparasion between a TSKgel UP-SW3000 column and a larger particle sized SEC column using the same operating mobile phase conditions showed that the TSKgel UP-SW3000 column has higher resolution. This suggest that the TSKgel UP-SW3000 column can be easily operated with an established method. The low backpressure allows TSKgel UP-SW3000 columns to be run in both HPLC and UHPLC systems. With the shorter column dimension that is available, faster analysis time can be achieved for increased sample throughput.

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